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Chapter 1

Formulation and Delivery of Proteins and Peptides

Design and Development Strategies

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The success of most peptide and protein drugs is dependent upon the delivery of the biologically active form to the site of action. In the design and development of formulations to achieve this goal, the formulation scientist must consider the clinical indication, pharmacokinetics, toxicity, and physicochemical stability of the drug. The development of a stable formulation is a necessary step for each new protein or peptide therapeutic. The degradation pathways and their impact on stability should be systematically analyzed and competing degradation rates must be balanced to arrive at the most stable formulation possible. Several routes of administration should also be considered and future development of new formulations may expand the number of potential options. Formulations for each route of administration may be unique and, therefore, have special requirements. In the case of depot formulations, there are many potential matrices, each of which has distinct characteristics that affect its interactions with the drug and its behavior *in vivo*. The formulation characteristics may have a dramatic impact on the *in vivo* stability of the drug as well as the pharmacokinetics and pharmacodynamics. The optimization of formulations, the routes of delivery, the design of depot systems, and the correlation between physicochemical stability and *in vivo* behavior are discussed in detail with recent examples. For new biotechnology-derived drugs including nucleic acids (DNA vectors and antisense RNA) to reach commercialization, all of the issues involved in the design and development of a drug formulation must be considered at an early stage of the overall development process.

Many aspects of biopharmaceutical process development have been well studied over the past twenty years. Difficulties in fermentation, cell culture, and, to some extent, purification and recovery have largely been overcome and these process steps have been well characterized for the production of many protein pharmaceuticals. However, one important field lags behind these others in its development. The design and production of protein and peptide drug formulations is not well developed and many of the mechanisms for stabilization and delivery of these drugs have not been

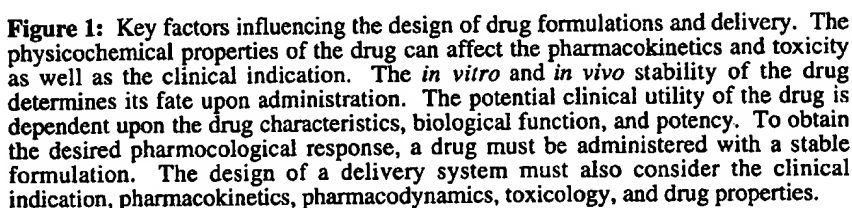
determined. In many cases, companies may initially neglect formulation and stability issues, resolving to simply store proteins or peptides in phosphate buffered saline or other solutions that have not been optimized for stabilizing the drug. Several unknowns still exist when developing a stable dosage form for peptides and proteins. Each molecule has its own unique physical and chemical properties which determine its *in vitro* stability. The formulation scientist must also be concerned about the *in vivo* stability of the drug. Thus, the development of successful formulations is dependent upon the ability to study both the *in vitro* and *in vivo* characteristics of the drug as well as its intended application.

Effect of Formulation Design and Delivery on Drug Development

As shown in Figure 1, a formulation scientist is confronted with a complex decision in choosing a formulation for delivery of a therapeutic protein or peptide. In the literature, the most common discussions of protein and peptide formulations focus on the physicochemical stability of these molecules. Indeed, the properties of the drug molecule are critical in determining the appropriate formulation for successful delivery and stability. The vast majority of the literature on protein and peptide formulations describes the degradation pathways for the drug. Many degradation pathways have been well characterized and, in some cases, degradation may often be predicted from the primary sequence of the protein or peptide (see 1 for examples). Once the formulation scientist has found a set of conditions that provide extensive stability (>2 year shelf-life), the formulated drug is tested in animal models for toxicity and pharmacokinetics. In many cases, this testing phase does not occur until the drug has moved from research into development. At this stage, many problems can occur including poor bioavailability due to the instability of the drug *in vivo*, rapid clearance, or the distribution of the drug in the body. Furthermore, an attempt is often made to resolve these difficulties by administering excess drug to achieve the desired biological effect. However, excessive drug doses often lead to toxicity problems. By this stage, the development of the drug has reached a critical decision point. The tendency in most organizations is to reconsider the development of the drug, sometimes resulting in the 'death' of the development project. However, the formulation scientist has the unique opportunity to work with the scientists in pharmacokinetics and toxicology to 'save' the development of the drug. By altering the formulation or the route of delivery, a drug can often have another opportunity to reach the stage of an Investigational New Drug (IND) filing. Unfortunately, the formulation scientist may not become involved until the drug has already encountered difficulties in animal studies. Thus, it is essential for the formulation scientist to work closely with the discovery research team, the pharmacokinetics department, and the toxicology department prior to the decision to move the drug into full scale development.

After all the difficulties are resolved in the early development stages, many protein and peptide drugs can still encounter problems in the clinic. The major clinical hurdles may be similar to those observed in the pre-IND animal studies. However, the company may have filed an IND for a therapeutic indication that will encounter complex formulation and delivery problems. The route and frequency of administration and the bioactivity or potency of the drug in humans are critical issues that are often not addressed in the pre-IND animal studies. If difficulties in delivery or potency of the drug arise during clinical trials, the formulation scientist along with others on the development team must reconsider the design of both the drug formulation and the clinical plan. These pitfalls may often be avoided by testing the drug in a suitable animal model, if available, and an extensive analysis of the patient population including a marketing survey of the end users (physicians, nurses, and/or patients). By establishing early in the development stage (e.g. between research and Phase I clinical trials) the best route and formulation for the drug, the potential for a

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clinically successful product and, ultimately, a marketed product increases dramatically.

The best route for delivery of a protein or peptide drug is often not investigated during the research stage or early in development. The protein or peptide is commonly administered systemically through an intravenous (i.v.) injection in initial animal testing. Thus, for indications that require a high local dose of the drug at the target site, high drug doses are required by i.v. injections. Due to toxicity problems, the efficacious dose may not be reached via i.v. administration. More recently, alternative routes of delivery have been studied. In particular, the therapeutic protein, recombinant human deoxyribonuclease I (rhDNase), must be delivered directly to the lung of cystic fibrosis patients to degrade the DNA in the mucus. rhDNase delivered systemically would clearly have little effect on the target site. While this example is an obvious candidate for an alternate delivery route (aerosol delivery of rhDNase), many other proteins and peptides may also benefit from alternative routes of delivery for therapeutic or clinical reasons. It is therefore essential to investigate the site of action and assess any side effects before choosing a route of administration.

In addition, when companies are developing competitive products, the future sales of the product may rest upon the superior formulation and delivery of the drug, assuming that the efficacy of the competing products are similar. For example, many existing therapeutic proteins such as human growth hormone and insulin are administered chronically requiring daily injections. Competitors with superior drug formulations that release a sustained level of the protein and, thus, require less frequent injections would dominate the market. An example of competing products is the development of sustained release formulations for a luteinizing hormone-releasing hormone (LHRH) agonists. Takeda Pharmaceuticals developed an LHRH agonist (leuprolide acetate) - polylactide-coglycolide formulation that could be administered monthly and provided a continuous sustained therapeutic level of LHRH for one month (2-5). This product, Lupron Depot®, had a ¥57 billion (~\$570 million) market in 1992 for prostate cancer, precocious puberty and endometriosis indications and competition from other types of LHRH agonist formulations, including daily injections and daily nasal delivery, have been insignificant (6). Similar competitive products also consist of controlled release systems using polylactide-coglycolide with different LHRH agonists (goserelin acetate, Zoladex®, 7, triptorelin, Decapeptyl®, 8). Overall, the clinical administration, patient compliance, pharmacokinetics, toxicity, and physicochemical properties of the drug must be considered to successfully develop a pharmaceutical protein or peptide drug.

Formulation Development Considerations

While development of novel delivery routes or systems is often necessary, the first step in development of any protein or peptide drug formulation involves the complete characterization of the drug properties and its stability in different formulations. Typically, a formulation scientist will begin by considering the physicochemical properties of the protein such as the isoelectric point, molecular weight, glycosylation or other post-translational modification, and overall amino acid composition. These properties along with any known behavior of the drug in different solutions (e.g. different buffers, cofactors, etc.) as well as its *in vivo* behavior should guide the choice of formulation components for testing in the initial screen of candidate formulations. The potential candidate formulations are composed of U. S. Food and Drug Administration (FDA) approved buffer components, excipients, and any required cofactors (e.g. metal ions). Often, the first choice of candidate formulations is based upon the previous experience of the formulation scientist with other proteins

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or peptides and, in many cases, a simple phosphate buffered saline solution may be one of the initial candidates.

A simplified approach to formulation development may proceed through the steps depicted in Figure 2. After obtaining all the available background information, one often evaluates several parameters in the initial screen of candidate formulations. One parameter that impacts all the major degradation pathways is the solution pH. Thus, the initial formulations also assess the pH dependence of the degradation reactions and the mechanism for degradation can often be determined from the pH dependence (9). The formulation scientist must quickly analyze the stability of the protein in each solution. Rapid screening methods usually involve the use of accelerated stability at elevated temperatures (e.g. 40° C; see references 10-13 for discussions of elevated temperature studies). Unfortunately, the FDA will only accept real time stability data for shelf life and accelerated stability studies may only serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature. The degradation of the protein for both accelerated and real time studies is then followed by assays developed for analysis of degradation products (see reference 14 for detailed review). The most common degradation pathways for proteins and peptides are listed in Table I. Several recent reviews have analyzed these pathways as well as potential methods to prevent degradation (11, 15-18). In each case, the amount of degradation must be minimized to achieve greater than or equal to 90% of the original drug composition after 2 years (e.g. $t_{90} \geq 2$ years). The FDA usually requires that a pharmaceutical product is not more than 10% degraded and the company must demonstrate that the degradation products do not have any adverse effects on the safety or efficacy of the drug. Many proteins and peptides can degrade extensively without effecting either their safety or efficacy. For example, 70% deamidated recombinant human growth hormone (rhGH) is fully bioactive and non-immunogenic, but this extent of degradation is not acceptable by regulatory agency standards for a therapeutic protein (19). The effect of degradation on the safety and efficacy of a protein or peptide is difficult to ascertain without extensive testing. Thus, the more conservative standards of the FDA and other regulatory agencies may often provide a less expensive alternative if a stable formulation (> 2 year shelf-life) can be developed.

To fulfill the regulatory requirements for a stable formulation, the scientist must consider all of the major degradation routes and the potential conditions for optimization. In the case of aggregation, the addition of surfactants or sugars can prevent denaturation events that lead to irreversible aggregation. If the deamidation rate is the dominant degradation route, the use of amine buffers such as Tris, ammonium, or imidazole may slow the deamidation. Alternatively, a reduction in pH will also decrease the deamidation rate, but the reduced pH may also lead to cleavage or cyclization at Asp-X residues where X is usually a residue with a small side chain (e.g. Gly or Ser) and this degradation has been observed in several proteins (1). Proteins with Asp-X degradation must then be placed in a higher pH buffer to avoid cleavage or cyclization. High pH conditions (> pH 8) will however catalyze oxidation, thiol disulfide exchange, and β -elimination reactions. These degradation pathways may be inhibited by the addition of free radical and thiol scavengers such as methionine. In addition, the method used to prevent one type of degradation may influence another degradation pathway. For example, by adding surfactants or other polymers to prevent aggregation, the residual peroxide in the surfactant may cause a more rapid oxidation (20). In some cases, the formulation pH must be reduced to decrease the rate of deamidation. Reducing the pH may also alter the solubility of the protein since many proteins have isoelectric points at or near the optimal pH (pH 5-6) for minimizing the deamidation rate. For each protein formulation, all the degradation pathways must be evaluated and often a balance must be achieved between the different degradation pathways.

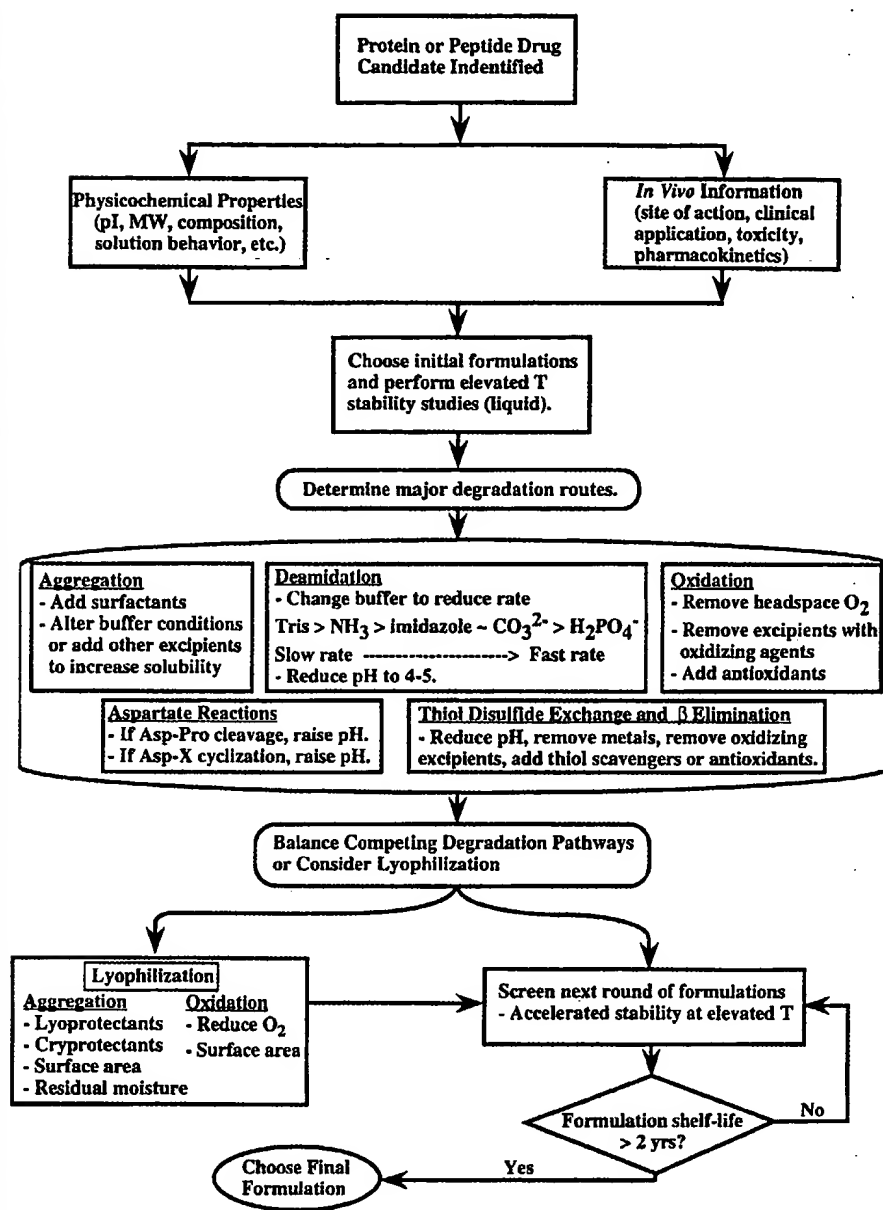


Figure 2: Simplified process diagram for formulation development (See Table I and text for detailed discussion).

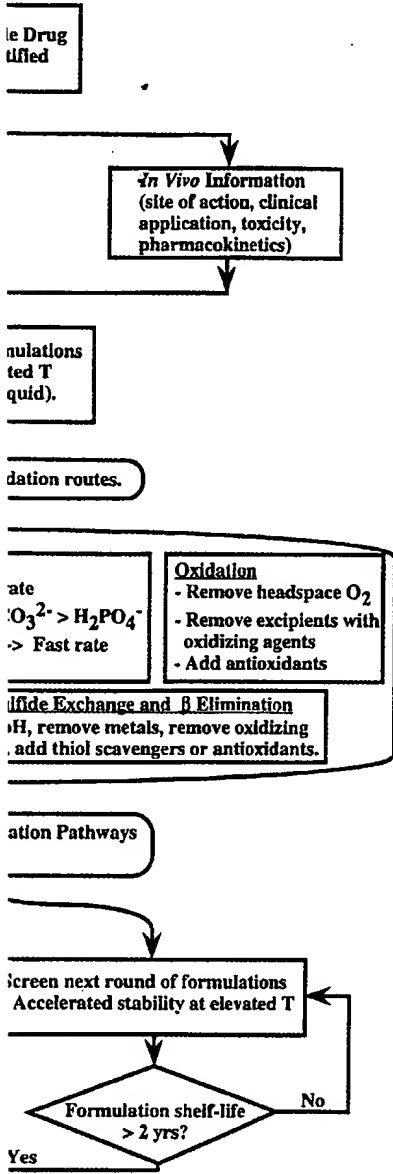


Table I: Common Degradation Routes for Proteins and Peptides in Aqueous Solutions ^a

Degradation Route	Region Effected/Results	Major Factors
Aggregation	Whole protein; reversible or irreversible self-association	Shear, surface area, surfactants, pH, T, buffers, ionic strength
Deamidation	Asn or Gln; acidic product, isoform, or hydrolysis	pH, T, buffers ionic strength
Isomerization/ Cyclization	Asn-X, Asp-X (X= Gly or Ser); basic product	pH, T, buffers ionic strength
Cleavage	Asp-X; fragments (proteolysis also possible from trace proteases)	pH, T, buffers
Oxidation	Met, Cys, His, Trp, Tyr; oxidized forms	Oxygen (ions, radicals, peroxide), light, pH, T, buffers, metals, (surfactants), free radical scavengers
Thiol Disulfide Exchange	Cys; mixed disulfides: intermolecular or intramolecular	pH, T, buffers, metals, thiol scavengers
β Elimination	Cys; dehydroalanine, free thiol	pH, T, buffers, oxygen (ions, radicals, peroxide), metals

^a This table lists degradation pathways commonly observed for proteins and peptides. However, this list is not comprehensive and many of these degradation routes may occur independently or in combination with one another.

Formulation development (See Table I and

The formulation scientist also has the option of developing a solid formulation such as a lyophilized powder. The removal of excess water from the formulation minimizes the degradation rates for deamidation and hydrolysis. The residual moisture in a solid protein or peptide formulation can contribute to the physical stability of the protein by preventing its denaturation and subsequent aggregation upon reconstitution. Recent studies on lyophilization of proteins have shown that in the absence of excipients, proteins require some residual water, usually referred to as bound water, for stability (21). In the presence of excipients such as sugars, the amount of remaining water may often be reduced to levels below the hydration layer (22). Two different theories are currently used to explain the excipient stabilization of proteins and peptides in the lyophilized state. The first theory is based upon the observed differences in crystallinity for each excipient in the dry state. The major differences in each excipient have been correlated to their glass transition temperature (23). This theory neglects the specific interactions between excipients during drying and in the final dried state. The second theory, often referred to as the water replacement hypothesis, contends that some excipients can substitute for water in the dried state and thereby provide stabilization (24, 25). However, this theory also has its faults since many excipients with similar hydrogen bonding characteristics provide different degrees of stabilization (e.g. mannitol versus trehalose). Recent work by Carpenter and coworkers has demonstrated that there may be two distinct mechanisms for excipient stabilization. These mechanisms include protection during freezing (cryoprotectants) and drying (lyoprotectants) (26). The specific interactions between proteins and excipients with these properties have not yet been determined, but it is probable that an understanding of these interactions will lead to more rational design of solid protein and peptide formulations. While these studies focus on the prevention of denaturation and aggregation of proteins in the lyophilized state, other degradation routes are also critical for solid protein and peptide formulations. For example, residual oxygen head space in a vial containing the solid formulation can affect the rate of oxidation (22). Therefore, the degradation issues for lyophilized formulations are comparable to liquid formulations, but deamidation and hydrolysis rates are usually slower in the solid state.

Once the decision has been made to narrow the formulation candidates or proceed with a lyophilized formulation, another round of screening at elevated temperatures is usually performed. If these studies account for the potential differences between real time data at the actual storage conditions (usually 2-8°C) as described elsewhere (10-13), then the formulation scientist can select two or three final candidate formulations that should have a greater than 2 year shelf-life for real time stability studies. Unfortunately, the formulation scientist is often asked to have a stable formulation within a very short time (< 6 months). Thus, the real time stability data is often limited, but these data are required for the IND filing since the FDA will not accept any accelerated stability data. Stability problems encountered later are treated as amendments during the clinical trials. Further changes after the IND filing may include scale-up of the manufacturing process including the formulation. The scale-up of formulations may include new processing and storage containers, bulk filling equipment, and new delivery systems or vial configurations. These modifications may require another evaluation of the formulation with additional optimization. Hopefully, any necessary changes in the formulation already in clinical trials will not alter the *in vivo* characteristics of the drug (e.g. clearance, immunogenicity or potency).

The Crucial Steps in Delivery

While considering the alternatives for formulating a protein or peptide drug, the formulation scientist must also consider the route of administration. As mentioned

of developing a solid formulation such as excess water from the formulation and hydrolysis. The residual water can contribute to the physical degradation and subsequent aggregation of proteins have shown that in the residual water, usually referred to as bound water, is referred to as the hydration layer. The first theory is based upon the excipient stabilization theory. The major theory, often referred to as the water theory, often referred to as the water theory, can substitute for water in the formulation (24, 25). However, this theory also has hydrogen bonding characteristics provide a mechanism for stabilization (e.g. sucrose versus trehalose). Recent work by others has indicated that there may be two distinct mechanisms include protection during drying (26). The specific interactions between excipients during drying are not yet been determined, but these interactions will lead to more rational formulations. While these studies focus on the proteins in the lyophilized state, other protein and peptide formulations. For formulations containing the solid formulation can be the degradation issues for lyophilized formulations, but deamidation and hydrolysis

the formulation candidates or proceed with screening at elevated temperatures is the potential differences between real time (2-8° C) as described elsewhere (10-15) or three final candidate formulations for real time stability studies. When asked to have a stable formulation, the real time stability data is often not filed since the FDA will not accept data encountered later are treated as changes after the IND filing may include the formulation. The scaling and storage containers, bulk filling configurations. These modifications in relation with additional optimization. Formulations already in clinical trials will not (e.g. clearance, immunogenicity or

previously, the route of administration can often be a critical variable in determining the success of the final product. As shown in Table II, there are many potential routes for the delivery of protein or peptide drugs. For proteins, only the direct injection and pulmonary routes of delivery have been approved by the FDA. Due to their large size, susceptibility to proteolytic degradation and requirements for an intact tertiary structure, proteins are difficult to deliver by oral, topical, or transdermal routes. Oral delivery of peptides and proteins usually results in low bioavailability (< 10%) and attempts to make stable prodrugs of peptides, which are more readily absorbed have provided some increase in the bioavailability (28). Unlike oral delivery, topical and transdermal delivery routes may be efficient enough for a localized treatment of skin disorders or diseases and, perhaps, for other localized therapies. Another method for protein delivery is the use of depot systems. Several companies are investigating their potential application. The depot systems offer the opportunity to localize the drug to the target site, reduce the frequency of injections, extend the half-life of the drug, and enhance its *in vivo* stability. Unfortunately, these systems also have inherent disadvantages that have not yet been overcome for their successful development into commercial products. A major disadvantage is often the use of harsh conditions (e.g. organic solvents or high temperature) to produce the depot, resulting in denaturation of the protein (29). In addition, for a long term depot formulation, the protein must be stabilized in an aqueous environment under physiological conditions at 37° C. Significant degradation has been observed for proteins stored under conditions analogous to those encountered in the depot (30, 31). For most of the novel delivery routes or depot formulations, the environment at the site of administration must be considered and stability studies of the drug in the same environment (e.g. serum) should be performed to assure that complete degradation does not occur before the drug reaches the desired site of action (32). Thus, the successful development of these formulations requires the initial development of protein formulations that are stable to both the process conditions and the *in vivo* environment.

Design of Depot Systems for Delivery. After the development of a formulation that meets these criteria, the material for the depot system must be chosen. The material should be biodegradable, well characterized, and nontoxic. The depot should also not alter the pharmacological properties of the drug and should act only as an inert carrier. Several examples of materials tested for depot systems are listed in Table III. While the materials derived from natural sources may often be more biocompatible, these materials may vary in their physical characteristics and can be difficult to obtain in a highly purified form. Many of the natural materials are derived from recombinant or animal sources and may contain contaminants such as endotoxins. In addition, some natural materials such as collagen or other proteins may invoke an unwanted immune response. In contrast, the synthetic materials are usually well characterized, highly pure polymers. The synthetic polymers have different physical and chemical characteristics. The polyanhydrides, polyesters, polyiminocarbonates, and polycaprolactones require the use of organic solvents or high temperatures for drug formulation. These systems are however well characterized and can be made reproducibly. On the other hand, the hydrogels, polyamino acids, and polyphosphazenes can be used to encapsulated drugs in an aqueous environment without organic solvents or elevated temperatures. These polymers are usually less stable and additional development is required to completely characterize their properties for controlled release of proteins and peptides. Of the synthetic materials, only the polyesters, specifically polylactic-co-glycolic acid (PLGA) and polylactic acid (PLA), are currently used in commercial depot systems, Lupron Depot® (2-5) and Zoladex® (58, 59). These products were recently approved as alternate formulations and the polymer matrix, PLGA, has been well characterized and has been used extensively in humans. These polymers have been used for over twenty

lating a protein or peptide drug, the route of administration. As mentioned

Table II: Routes of Delivery for Proteins and Peptides

Delivery Routes	Formulation and Device Requirements	Commercial Products ^a
Invasive		
Direct Injection	Liquid or reconstituted solid, syringe	
intravenous (i.v.)		Activase [®]
subcutaneous (s.q.)		Nutropin [®]
intramuscular (i.m.)		RecombiVax [®]
intracerebral vein (i.c.v.)		
Depot system	Biodegradable polymers, liposomes permeable polymers (not degradable), microspheres, implants	
LHRH analogs (s.q. or i.m.)		Lupron Depot [®] Zoladex [®] Decapeptyl [®]
Noninvasive (see reference 27 for review)		
Pulmonary	Liquid or powder formulations, nebulizers, metered dose inhalers, dry powder inhalers	Pulmozyme [®]
Oral	Solid, emulsion, microparticulates, absorption enhancers	
Nasal	Liquid, usually requires permeation enhancers	Synarel [®]
Topical	Emulsion, cream or paste (liposomes)	
Transdermal	Electrophoretic (iontophoresis), electroporation, chemical permeation enhancers, prodrugs, ultrasonic	
Buccal, Rectal, Vaginal	Gels, suppositories, bioadhesives, particles	

^a Nutropin[®] (recombinant human growth hormone), Activase[®] (recombinant human tissue plasminogen activator), and Pulmozyme[®] (recombinant human deoxyribonuclease I) are all products of Genentech, Inc. RecombiVax[®] (recombinant Hepatitis B surface antigen) is produced by Merck & Co. Lupron Depot[®] (leuprolide acetate - PLGA) is a product of Takeda Pharmaceuticals. Zoladex[®] (goserelin acetate - PLGA) is produced by the Imperial Chemical Industries, Ltd. Decapeptyl[®] is a manufactured by Debiopharm. Synarel[®] (nafarelin acetate) is made by Syntex Corporation.

Proteins and Peptides

Device	Commercial Products ^a
tuted solid, syringe	Activase® Nutropin® RecombiVax®
olymers, liposomes ers (not degradable), olants	Lupron Depot® Zoladex® Decapeptyl®
formulations, d dose inhalers, rs	Pulmozyme®
microparticulates, ers	
quires permeation	Synarel®
or paste (liposomes)	
ontophoresis), hemical permeation gs, ultrasonic	
s, bioadhesives, particles	
one), Activase® (recombinant human ® (recombinant human deoxyribo- RecombiVax® (recombinant rck & Co. Lupron Depot® ceda Pharmaceuticals. Zoladex® Imperial Chemical Industries, Ltd. n. Synarel® (nafarelin acetate) is	

Table III. Biodegradable Materials for Controlled Delivery of Proteins or Peptides

Materials	Degradation Mechanism	Reference ^a
Natural		
Starch	Amylase	33, 34
Alginate ^b	pH, Enzymes	35, 36
Collagen (Gelatin)	Collagenase	37-39
Proteins (Cross-linked albumin)	Enzymes	40, 41
Tricalcium phosphate ^c or calcium carbonate (hydroxyapatite)	Dissolves over time	42-44
Synthetic		
Hydrogels	Chemical or enzymatic hydrolysis, solubilization in aqueous media	45
Polyanhydrides	Hydrolysis	46-48
Polyesters (polylactides)	Ester hydrolysis, esterases	49, 50
Poly (ortho esters)	Ester hydrolysis, esterases	49
Polyiminocarbonates	Hydrolysis	51, 52
Polycaprolactones	Hydrolysis	53, 54
Polyamino acids	Enzymes	55
Polyphosphazenes ^b	Hydrolysis, dissolution	56, 57

^a Reviews describing the application of these materials for delivery of proteins or peptides. When recent reviews were not available, several examples were listed.

^b Alginate polyphosphazene depot systems usually require a cross-linking agent such as calcium or polycations.

^c These depot systems are often used for mechanical strength in bone formation and are usually used in combination with another carrier such as collagen or starch.

years in resorbable sutures and have well established Drug Master Files at the FDA. In addition, PLGA and PLA are readily degraded in water to lactic and glycolic acid by ester hydrolysis. They are also available in a range of compositions and molecular weights from commercial suppliers. Several studies have been performed to determine the *in vitro* and *in vivo* characteristics of these polymers and their release of proteins and peptides (5, 60, 61). The *in vivo* degradation and injection site histology of several PLGA and PLA preparations have also been studied in animals (62-64). These studies indicated that the polymer did not cause any abnormal inflammation or irritation and the surrounding tissues completely recovered without scarring after degradation of the polymer. Thus, while the other materials may offer some advantages in manufacturing or *in vivo* performance, these polymers will probably be used for the first commercially available, injectable depot system containing a protein.

The polylactides have been used to develop protein or peptide depot systems for different applications ranging from bone formation to vaccines. Several recent examples of PLGA or PLA formulations containing proteins or peptides are listed in Table IV. For bone indications, the formulations were used to maintain a local concentration of the protein at the site while another material such as calcium carbonate was used for mechanical strength (65, 66). Since peptide - PLGA formulations are proven products, many of the peptide indications may result in PLGA formulations for chronic therapies (67-71). Finally, the vaccine - PLGA formulations may ultimately provide a route for single administration of vaccines which are currently given in multiple doses (72-76). These new vaccine formulations would eliminate the common problem of the patient's failure to return for booster immunizations, especially in large urban areas with transient populations and underdeveloped countries. In addition to these applications, new methods of preparation and derivatives of the polylactides may provide new routes of administration. For example, clearance of the drug may be reduced by encapsulating the protein in PLGA nanospheres with PEG attached to their surface. PEG would act to shield the microspheres from clearance by the reticuloendothelial system. These 'stealth' nanospheres may allow for the continuous release of proteins and peptides due to the increased blood circulation half life (79). Overall, the polylactides may provide a more efficient method for delivery of drugs that require chronic or repeated administration.

Other potential depot delivery systems such as liposomes may also be able to reduce the administration frequency of drugs and provide improved *in vivo* drug characteristics. Liposomes may act as inert carriers of proteins, peptides, and DNA or RNA for gene therapy or antisense technology, respectively. While there are a few commercial liposome drug formulations on the market, these formulations do not consist of a protein (80-82). However, liposomes may find utility in solubilizing lipophilic peptides or proteins and as a result of their large size, they may act as a depot for the drug, preventing clearance or degradation. A protein or peptide is often presented on the surface of the liposome and can thus bind to its receptor or perform its biological function (83). Many of these formulations may only increase the duration of response for a few hours. The composition of liposomes directly impacts the amount of time that the liposome remains in the circulation. The common components of a liposome are phospholipids, cholesterol, and stabilizing components such as polyalcohols or sugars for protection during processing. By altering the liposome composition, the duration of bioactivity can be extended to several days. This effect was demonstrated by utilizing Stealth® liposomes and vasopressin (84). Stealth® liposomes consist of lipids with polyethylene glycol (PEG) covalently attached to their hydrophilic head groups. These PEG molecules act as a shield to prevent uptake by phagocytic cells in the reticuloendothelial system. Liposomes may

lished Drug Master Files at the FDA. led in water to lactic and glycolic acid a range of compositions and molecular al studies have been performed to s of these polymers and their release of legradation and injection site histology also been studied in animals (62-64). t cause any abnormal inflammation or tely recovered without scarring after he other materials may offer some nance, these polymers will probably be njectable depot system containing a

protein or peptide depot systems for mation to vaccines. Several recent ining proteins or peptides are listed in tions were used to maintain a local le another material such as calcium i (65, 66). Since peptide - PLGA he peptide indications may result in '-71). Finally, the vaccine - PLGA for single administration of vaccines -76). These new vaccine formulations patient's failure to return for booster reas with transient populations and these applications; new methods of tides may provide new routes of drug may be reduced by encapsulating ached to their surface. PEG would act he reticuloendothelial system. These uous release of proteins and peptides : (79). Overall, the polylactides may drugs that require chronic or repeated

liposomes may also be able to reduce d provide improved *in vivo* drug riers of proteins, peptides, and DNA or , respectively. While there are a few ie market, these formulations do not omes may find utility in solubilizing of their large size, they may act as a adation. A protein or peptide is often n thus bind to its receptor or perform formulations may only increase the position of liposomes directly impacts as in the circulation. The common olesterol, and stabilizing components during processing. By altering the vity can be extended to several days. lth® liposomes and vasopressin (84). olyethylene glycol (PEG) covalently ese PEG molecules act as a shield to oendothelial system. Liposomes may

Table IV. Partial List of Recent Examples Using Polylactides for Controlled Release of Proteins or Peptides

Protein or Peptide	Polymer ^a	Application	Reference
Bone morphogenetic protein	PLA-PEG copolymer (650 Da PLA - 200 Da PEG)	Bone formation	65
Transforming growth factor- β_1	50:50 PLGA (40-100 kDa) (+ demineralized bone matrix)	Bone formation	66
Thyrotropin	75:25 PLGA (11 kDa)	Central nervous system dysfunction	67
Growth hormone releasing factor	75:25 PLGA (91 kDa)	Growth hormone deficiency	68
Somatostatin analogue	55:45 PLGA (23-76 kDa)	Acromegaly, tumors	69
Neurotensin Analogue	PLA (2-6 kDa)	Psychotropic	70
Cyclosporin A	50:50 PLGA (0.44 & 0.80 dL/g)	Immunosuppression	71
Colonizing factor antigen (<i>E. coli</i>)	PLGA (0.73 dL/g)	Oral vaccine	72
Cholera toxin B subunit	PLA (2 kDa)	Oral vaccine	73
Diphtheria toxoid formilin treated	PLA (49 kDa)	Vaccine	74
Ovalbumin	50:50 PLGA (22 kDa) 85:15 PLGA (53 kDa)	Vaccine	75
Tetanus toxoid	50:50 PLGA (100 kDa)	Vaccine	76
LHRH antagonists	50:50 PLGA 75:25 PLGA	Tumor suppression	77
Horse radish peroxidase Bovine serum albumin	75:25 PLGA (10 kDa)	Marker proteins Mechanistic studies	78

^a X:Y PLGA indicates the mole fraction (%) of lactide (X) and glycolide (Y) in the copolymer. The polymer size is reported as either molecular weight in kilodaltons (kDa) or intrinsic viscosity in decaliters/gram. Some references did not provide complete descriptions of the polymer.

also have utility for presentation of antigens and adjuvants. Liposome presentation of proteins may mimic the normal antigen - membrane configuration and the liposome will be consumed by phagocytic cells. This approach has been applied to the development of vaccine formulations by using dehydration-rehydration vesicles (DRVs) which trap protein on their surface during the drying process (85). In addition, it may be possible to target liposomes to specific cell populations by placing a marker protein such as a monoclonal antibody or cytokine on the surface of the liposome (86, 87). Thus, liposomal formulations may provide unique methods for targeting and delivery of proteins and peptides.

The Role of Pharmacokinetics in Delivery. When choosing the type of delivery system and the route of administration, the pharmacokinetics of the formulation must be considered. It may often be easier to predict the pharmacokinetics of a depot formulation which provides a sustained therapeutic level of the drug than a solution of the drug administered at different sites or in different formulations. The impact of the formulation on the pharmacokinetics of a drug are well illustrated in the case of insulin as shown in Table V. Insulin has the ability to form multimers of the native protein and these multimers (zinc-hexamer) can reversibly form crystals. After subcutaneous injection, the multimers must dissociate into monomers to diffuse across the capillary membrane and bind to the receptor. By formulating insulin in different solutions, the pharmacokinetics are dramatically altered. The differences in time before the insulin reaches therapeutic levels and the duration of its therapeutic effect can result in severe consequences if the incorrect formulation is administered to a patient. In particular, a patient with severe side effects from hyperglycemia may require a rapid onset formulation and may be seriously injured if administered a slower acting form. In addition to the differences in pharmacokinetics due to the associated state of the protein, each formulation has a different stability (88, 90, 91). For example, the presence of rhombohedral crystals and free zinc can accelerate hydrolysis (88). The dominant degradation mechanism is hydrolysis in all the formulations except the isophane solutions where the formation of high molecular weight aggregates occurs at a much greater rate. These formulations also invoke a higher incidence of antibodies to insulin as well as the additive, protamine (90). The degradation of insulin by the solution conditions (additives, pH, etc.) is the result of the conformational flexibility of the insulin monomer (91). In the case of insulin, the formulation has an enormous impact on the stability and the pharmacokinetics of the drug, both of which determine its ultimate application. Many other protein and peptide drug formulations may directly alter the pharmacokinetics and pharmacodynamics of the molecule as well as its *in vitro* stability (92, 93).

Conclusions and Future Directions

The design and development of formulations and methods of delivery for proteins and peptides is dependent upon several variables. The relationship between the formulation, route of delivery, pharmacokinetics, toxicity, and clinical indication must be carefully balanced to successfully develop a protein or peptide drug. A formulation scientist should consider the possible options for each new therapeutic entity prior to the design of a formulation. Many new systems such as polymers and liposomes may offer attractive alternatives to traditional liquid or solid dosage forms. While these new delivery options often require invasive methods, development of new noninvasive delivery routes such as pulmonary or transdermal delivery may yield promising results for future applications. The formulation scientist will soon have to address new challenges in the delivery of DNA and RNA for gene therapy and antisense technology, respectively. The success of these future therapies may depend upon the ability to successfully deliver the nucleic acid polymer to the desired cell nucleus. Thus, the formulation scientist will play a key role in the advancement of these technologies. Further, the targeting of drugs to specific sites of action should

adjuvants. Liposome presentation of brane configuration and the liposome s approach has been applied to the ng dehydration-rehydration vesicles during the drying process (85). In to specific cell populations by placing dy or cytokine on the surface of the ons may provide unique methods for

When choosing the type of delivery macokinetics of the formulation must list the pharmacokinetics of a depot tic level of the drug than a solution of erent formulations. The impact of the ig are well illustrated in the case of bility to form multimers of the native can reversibly form crystals. After dissociate into monomers to diffuse receptor. By formulating insulin in amatically altered. The differences in ls and the duration of its therapeutic correct formulation is administered to side effects from hyperglycemia may : seriously injured if administered ances in pharmacokinetics due to the i has a different stability (88, 90, 91). crystals and free zinc can accelerate mechanism is hydrolysis in all the ere the formation of high molecular e. These formulations also invoke a as the additive, protamine (90). The is (additives, pH, etc.) is the result of omer (91). In the case of insulin, the bility and the pharmacokinetics of the plication. Many other protein and alter the pharmacokinetics and s *in vitro* stability (92, 93).

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Table V. Effect of Insulin Formulation on the Pharmacokinetics after Subcutaneous Injection (89, 90)

Trade Name ^a	Formulation	Pharmacokinetics ^b
Humulin® R Novolin® R	Zinc-insulin crystalline suspension (Acid regular)	Rapid onset, short duration Start: 0.5 hr; Peak: 2.5-5 hr End: 8 hr
Humulin® N Novolin® N	Isophane suspension protamine, zinc crystalline insulin (buffer water for injection)	Intermediate-acting, slower onset, longer duration than regular insulin Start: 1.5 hr; Peak: 4-12 hr End: up to 24 hr
Humulin® 70/30 ^c Novolin® 70/30	70% isophane suspension 30% zinc crystalline	Intermediate-acting, faster onset, longer duration Start: 0.5 hr; Peak: 2-12 h End: up to 24 hr
Humulin® U	Extended zinc-insulin suspension - all crystalline	Slow-acting, slow onset, longer, less intense duration than R or N forms.
Humulin® L Novolin® L	70% zinc-insulin crystalline suspension, 30% amorphous insulin (cloudy suspension)	Intermediate-acting, slower onset, longer duration Start: 2.5 hr; Peak: 7-15 hr End: 22 hr
Humulin® BR	Zinc crystalline insulin dissolved in sodium diphosphate buffer	Rapid onset, short duration Use in pumps only.

^a Humulin® products are recombinant human insulin derived from *Escherichia coli* and produced by Eli Lilly & Company. Novolin® products are recombinant human insulin derived from *Saccharomyces cerevisiae* and produced by Novo Nordisk. Both companies also sell other forms of recombinant human insulin and may have additional forms (formulations or new drugs) in clinical trials.

^b The pharmacokinetics of each formulation may vary greatly among different individuals. The values reported here are taken from reference 89. The onset of therapeutic levels of insulin is referred to as the start of the effect. The maximum serum level of insulin is denoted as the peak and the time at which the insulin levels are below therapeutic levels is listed as the end of the therapeutic time course.

^c Solution consists of 70% N form and 30% R form for both products.

increase the utility of many proteins and peptides that are potent and toxic. Low doses of these drugs can then be used and directed to the target site. Finally, future advances in the transport of proteins and peptides across biological barriers such as the blood brain barrier (94-99) and gastrointestinal epithelium (100-104) may result in new delivery options and successful therapies.

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